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Yu.N. Gorodilov and T.N. Svimonishvili

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RANGE OF TEMPERATURE TOLERANCE OF ATLANTIC SALMON DURING INCUBATION IN APPARATUSES WITH CLOSED WATER CIRCULATION

Yu.N. Gorodilov and T.N. Svimonishvili

In the breeding of salmon Salmo salar L., fish hatcheries in our |103 country use a water supply from sources with natural temperature regimes. The question of a controllable optimal thermal regime during the incubation period is still at the stage of discussion and preliminary analysis. Two lines of thought emerge in the investigation of this question. According to one, it is advisable to preserve the natural regime of incubation of salmon eggs, but this regime must be "corrected" in the autumn and spring periods when the water temperature is unstable. It has been found (Yandovskaya, 1967, 1976, Kazakov, 1971) that fish-hatchery indicators for alevins could be improved if the thermal regime in the autumn and spring periods of incubation were maintained at a higher level than the long-term average for these period in natural conditions.

To resolve this question, we took another approach, believing that it is necessary to investigate the limits of the tolerance of salmon embryos to temperature and, above all, to determine the optimal temperatures for their development. Knowing the range of tolerance to the temperature of incubation,

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we can make an a-priori assumption that the mean values of this range will correspond best to the desired conditions. Such an approach to finding the most favourable temperatures for the incubation period was used in a study of Pacific salmon and whitefish (Combs, 1965; Colby and Brooke, 1970; Hokanson et al., 1973; Brooke, 1975).

However, in solving the problem of the optimal regime of salmon incubation, it is not enough to limit ourselves only to the study of the most suitable conditions for realizing the biological program encoded in the cells of salmon embryos. Since salmon is a valuable species and its rearing entails large expenditures, the optimal incubation regime should take into account production considerations as well. We believe that three conditions should be observed in a comprehensive appraoch to this question. The optimal thermal regime during the incubation period of salmon should satisfy the following requirements: /104
1) it should ensure the normal development and the best survival rate of the embryos and should ensure the formation of downstream-migrant juveniles; 2) it should hasten the formation of juveniles, i.e. shorten not only embryogeny, but also the entire freshwater (hatchery) period of salmon life; 3) it should be technically simple and inexpensive in terms of energy consumption for maintenance of the required temperature.

Heating the water and maintaining its temperature at the required level is a technically uncomplicated problem, and appropriate devices have been developed to deal with it (Petrenko et al., 1972) which do not call for the reuse of the heated water. The warming up of the eggs for a short time at the beginning and end of the incubation period, which N.I. Yandovskaya and R.V. Kazakov (1974) propose for the propagation of salmon in hatcheries, will not entail high energy expenditures, and in the context of accelerating embryonic development and increasing the vegetative period, this may be economically advisable.

We know that salmon eggs can be incubated at a fairly high constant

temperature - up to 10-12^{o} (Hayes, 1949; Peterson et al., 1977). To heat the water to such a temperature during the entire incubation period and to use it once would be very expensive. Therefore, in order to study the temperature-tolerance range for salmon embryogeny, it is advisable to use incubators with closed water circulation and to use the water repeatedly. Such a method of supplying water is practised not only for incubating eggs, but also for rearing juvenile salmon (Burrows and Combs, 1968).

We have already been using apparatus with closed water circulation for incubating salmon eggs for over 10 years (Gorodilov, 1971). Data on the construction of these incubators are given in the book Apparatuses for Biological Development (1975). Within the incubation apparatus with closed water circulation one can establish any incubation regime and obtain data on the tolerant and optimal temperature zones for embryonic development. However, the practical use of this system should be preceded by a study of the dynamics of the accumulation of metabolic products released by the eggs and of their effect on the embryos, and by the determination of the rates of water expenditures and of the degree to which the water must be cleansed of metabolites. It is felt that the most toxic product released by the eggs is ammonia (Burrows, 1964), and it is proposed that the simplest way to lower its concetration is to replace part of the recirculating water (Fedorov, Smirnov, 1978). In order to answer some of the questions that have been raised, we studied the development of Atlantic salmon eggs at ten different constant temperatures, with the replacement of part of the recirculating water, in one series of experiments, and with no replacement of water in another series, over the course of the incubation period. The criteria for assessing the results of the incubation of the eggs were as follows: the survival rate of the embryos and alevins, the absolute and relative duration of a number of selected stages, the rate of development, the length and weight of the alevins at hatching, and their ability to change from endogenous feeding to exogenous feeding.

In this work, which analyzes the course of the embryogeny of salmon in relation to temperature, we examine the absolute duration of the same stages at different temperatures of egg incubation. As we know, this indicator varies in relation to temperature, and the data of the different experiments are difficult to compare. An unambiguous quantitative characteristic of a stage, independent of the complex of external conditions, can be provided by the measurement of its relative duration. This method of expressing quantitatively the stages of embryonic development, consisting of the fact that the duration of the stages /105 under consideration is correlated with the duration of a particular determined segment of development at constant environmental conditions is substantiated in detail in the work of T.A. Detlaf and A.A. Detlaf (1960); it is suggested that the time interval between the cellular cycles (τ_0) within a period of synchronous cleavage divisions be used as the standard determined segment. One can familiarize oneself with the use of this standard for measuring the relative duration of the developmental stages of embryos in the works of G.M. Ignat'eva (1970), T.A. Detlaf (1977), etc. The relative duration of any stage may be regarded as an indicator of the biological age of the embryo at this stage.

However, the method of measuring the interval has a serious drawback, which is that the moment at which the sulcus appears on the surface of the egg (the most available way of determining the duration of a cellular cycle) is difficult to grasp in essence by means of a qualitative criterion. We proposed to determine and to use, as a standard for measuring the relative duration of the stages of embyogeny, the formation time of one pair of somites. Our proposal is based on the fact that the rate of metamerization of the body of the embryo is constant, and the differentiation of the segments (pairs of somites) during this process occurs with one and the same time interval (Gorodilov, 1980). Because of this, a given time interval (τ_s) is readily calculated if at a constant temperature we establish the time of onset of the two stages with

a different number of somites at the beginning and end of the period of metamerization (Gorodilov, 1980). In the present work, in comparing the embryos from different regimes of incubation, we used precisely this method of assessing their biological age.

MATERIALS AND METHODS

Studies were conducted on the eggs of Atlantic salmon from the Kola and Neva rivers in 1973-1978. After fertilization and swelling, the eggs were packed in water in a cooling sac with ice and were delivered on the same day to the laboratory where they were placed in incubators, in which various constant temperatures were maintained. Usually, during a season, the development of eggs at 3-4 different temperature regimes was observed simultaneously. The eggs from one female or mixed from several females were divided into a number of parts - equal to the number of experiments, and one part was placed in each incubator, so that incubated material was uniform in all experiments. Let us add that we shall call all the eggs of a single fertilizing period a lot, and groups of eggs in one incubator from different females, and observed separately, placements.

Fig. 1 shows one of the incubators. The eggs are placed in the compartments of a cascade over which the water flows from an upper tank to a lower tank, constantly washing the eggs. The water is pumped to the upper tank.

The development of the salmon embryos was studied at different constant temperatures, from 1.0 to 11.6°C. The water in the incubators was circulated in two ways: with periodic replacement of part of the water (Table 1) and with the use of the same water during the entire period of incubation (Table 2). The Tables provide data on temperature, the load of the incubators and the water expended per egg for the entire incubation period. When the total amount of water being circulated is not over 130 L, the average rate of its exchange is maintained at a level of 2.4-2.7 L/min. When incubation occurred without /106 water replacement, only small amounts of water were added to make up for losses

due to spillage and evaporation, etc. Let us note that at a temperature of 1.0° the additions were considerable (80 L) because of the long duration of this experiment (230 days).

Fig. 1. Atlantic salmon eggs in a cascade incubator

In the experiments with water replacement, after the stage of the closing of the blastopore, 7-10 L of the water being circulated was replaced with fresh water 2-3 times a week. The water in the incubator was not reconditioned in any way.

Fluctuations in water temperature during a given regime did not usually exceed ±0.1°. The eggs in the cascade vessels, in which the water level was approximately at 1 cm, were arranged in a single layer. Because of the gradation and the large surface area of the cascade, a good level of oxygen concentration is maintained in the flowing water; our readings at the entrance and exit of the incubator at various stages of development was 11-12 mg O_2/L.

The temperature and rate of water flow were monitored every day during incubation, and if there were dead eggs, they were removed.

The development of the embryos was monitored on the live eggs. This was done by dissecting the membranes of at least 3-4 eggs in an isotonic saline solution (double concentration of Goltfreter's* medium) and by determining the stage of development of the embryos under a microscope (magnification 5 x 5) and establishing the apparent deviations from the normal course of embryogeny. The indices for their temporal and relative duration, beginning with the time of fertilization, were obtained for each phase. The measure of /107

*A transliteration. Tr.

relative duration of the stages and the entire embryogeny at a given temperature was the time required for the formation of one pair of somites (τ_s) at that temperature.

Table 1

Incubation of salmon eggs at various temperatures with replacement of water being circulated

Incubation temperature, °C	Number of eggs	Duration of incubation, days	Volume of water being circulated, L	Water replacement for incubation, L	Expenditure of water per embryo, m L
2,0	3500	167—172	90	900	280
3,1	6500	131—135	75	350	70
4,0	3200	120—123	75	260	105
5,0	10000	95—97	130	320	45
6,2	5800	79—81	85	250	60
9,0	6000	50—52	90	180	45
10,0	1100	43—45	30	140	155
11,6	600	—	—	—	—

Note: At a temperature of 11.6°, most embryos developed only to the yolk plug stage

Table 2

Incubation of salmon eggs at various temperatures without replacement of water being circulated

Temperature in incubator,	Number of eggs	Duration of incubation, L	Volume of water being circulated, L	Water added to make up losses for incubation, L	Expenditure of water per embryo, m L
1,0	7000	227—230	75	80	22
2,7	4300	158—160	75	20	21
4,0	6400	115—118	130	50	28
5,0	8600	95—97	130	25	18
7,2	4300	69—71	85	16	20
8,0	9000	57—59	85	45	14
9,1	5700	49—50	65	15	14

temperature. The value of τ_s was determined by calculation after the time of onset of the two stages was determined: t_1 with n_1 pairs of somites (not fewer than 1) and t_2 with n_3 pairs of somites (not more than 60). Then

$$\tau_s = \frac{t_2 - t_1}{n_2 - n_1}$$ (Gorodilov, 1980).

As an example, we shall calculate τ_s for a temperature of

2.7°. From the stages in which the somites were counted, we select the stages with the minimum and maximum number of somites, since the accuracy of determination of τ_s is greater, the greater the difference $n_2 - n_1$. For temperature 2.7°: on November 9 at 9 hr - 8 pairs of somites in 3 embryos and 9 pairs of somites in 6 embryos (an average of 8.7); on December 3 at 14 hr - 57 pairs in 7 embryos and 56 pairs in 1 embryo (an average of 56.9). Thus, in 581 hr, 48.2 pairs of somites are formed. Hence, τ_s equals 12 hr.

At the beginning of the peak of the hatching period, 25 embryos were preserved in a 40% solution of formalin for the purpose of measurement. The length of the alevins were measured with an accuracy of up to 0.5 mm, and the weight of their yolk and body with an accuracy of up to 0.1 mg; the percentage of yolk in the total alevin weight was determined. All comparisons of measurement and weight indicators were made only within a single lot of eggs since the size of the alevins can depend on the original size of the unfertilized eggs (Galkina, 1969).

In most experiments, after hatching, some of the alevins were put in vats for the transfer to exogenous feeding. They were first fed with the yolk and later with daphnia and alogochaetas.

RESULTS

Rate of development and duration of the embryonic stages of salmon at various temperatures

The rate of development of embryos can be determined by comparing the formation period of one pair of somites at different temperatures. This time /108
(τ_s) can easily be determined with sufficient accuracy, using the method described above. The formation time of one pair of somites at various temperatures, from 11.6 to 1.0° is given below.

Incubation temperature, °C	11.6	10.0	9.0	8.0	7.2	6.2	5.0	4.0	3.1	2.7	2.0	1.0
Duration, min	190	230	255	280	335	390	470	555	680	720	840	1020

The duration of this single act of morphogeny changes from 190 min at a temperature of 11.6^0 to 1020 min at 1.0^0, i.e., the rate of the process in one case is 5.4 times higher than in the other. It was important to determine whether the proportionality between the time of somite formation and other stages of embryogeny is preserved when the temperature of incubation changes. To this end, 10 stages were selected. They were well identifiable in terms of morphological characteristics, and their onset could be determined with an accuracy of up to several hours or days at the lowest temperatures. Table 3 provides the data on the time (rounded off to days) during which the embryos, after fertilization, reach each of the selected stages at 10 different temperatures. These data were obtained under strict observance of the constancy of thermal regime, and the stages were determined with as much accuracy as possible. It was found that if the stages of development are measured by the number of τ_s segments, then for each stage the duration will be expressed by the same number of τ_s (the fluctuations in these numbers are small when a stage is determined by quantitative characteristics), regardless of which temperature was chosen during incubation.

Thus, we have confirmation here of the possibility to using the interval of the formation time of one somite as an indicator of the biological growth of embryos.

The data in Table 3 show that in order to reach the beginning of gastrulation, 35-36 τ_s's are required, to reach the beginning of eye pigmentation - 120-125 (we regard this to be the stage when a pigment band appears and is visible under a magnifying glass in the upper part of the optic cup after the removal of the egg membrane), and to reach the emergence of the first alevins from the membranes - at least 280. Thus, development to the stage of gastrulation takes 0.12 of the total time of embryogeny and development to the beginning of eye pigmentation - 0.40 of the total time. The variability of the sum of τ_s during hatching corroborates the hypothesis stated earlier that the hatching

Table 3

Duration of some stages of embryonic development of salmon (in days) at various constant temperatures and age of embryos in these stages, expressed in relative units (τ_s)

Incubation temperature, °C	Duration τ_s, days	Beginning of gastrulation	5 pairs of somites	23 pairs of somites	45 pairs of somites	55 pairs of somites	Beginning of eye pigmentation	½ vascularization of yolk	somite processes in 3rd-7th caudal segments	Initiation of ventral fins	Hatching of alevins (0-50%)
10,0	0,16	6	10	13	16	18	20	23	28	34	43–45
9,0	0,18	6,5	10,5	14	18	20	22	26	31	38	50–52
8,0	0,20	7	12,5	15,5	20	22	24	28	34	45	56–58
7,2	0,23	8,5	14	18	23	25	28	34	41	49	66–69
6,2	0,27	9	16	20	27	30	33	39	47	57	79–81
5,0	0,33	12	20	26	33	36	40	48	59	70	93–95
4,0	0,39	14	23	30	38	42	46–47	54	68	81	110–116
3,1	0,47	17	28	37	48	53	58–59	–	84	100	132–135
2,0	0,58	21	35	46	59	65	73–75	—	101	125	165–170
1,0	0,71	26	43	55	70	79	88	100–101	125	150	218–225
Age from the beginning of fertilization (τ_s)		35–36	60	78	100	110	120–125	142–146	172–178	208–215	280–300 (at 10°–267–280 and at 1°–307–317

period is not confined to a definite morphological stage.

Table 3 combines the data on the development of Altantic salmon and Neva salmon. The differences between them in terms of duration τ_s and different stages of development were not established since all of the data presented here generally refer to Altantic salmon. The results of egg incubation based on the two methods of water recirculation, with and without water replacement, differed for Altantic salmon and Neva Salmon; therefore, it is advisable to examine them separately.

Incubation of Atlantic salmon eggs without replacement of the water being circulated

According to this method, the eggs were incubated at various temperatures ranging from 2.0 to 11.6°. For some parameters the eggs of the fish of these two populations cannot be compared since the eggs of Neva salmon are considerably larger than those of Atlantic salmon. In one season, we weighed 25 unfertilized preserved eggs, each, for Atlantic salmon and Neva salmon, and their average weight was 90.0 ±0.68 and 125.5±2.00 mg, respectively. This prompted us to present the data on Atlantic salmon and Neva salmon in separate sections.

Atlantic salmon. No alevins were obtained at a temperature of 11.6°. /110 The development in the early stages is outwardly normal. The value for τ_s was 190 min. At such a rate the stage of gastrulation already begins after 5 days, and the stage of 5 pairs of somites (approximately half of the yolk is overgrown with the blastomere) forms after 8 days. By this time, the mortality rate was negligible (under 1%), and the development of the embryos was fairly synchronous. The large-scale loss of eggs began in the yolk-plug stage (Fig. 2): over 80% of the embryos ceased to develop at this stage and soon died (turned white). Some of these eggs were preserved, and the embryos from them were treated histologically. It was ascertained that development ceased at the stage of the formation of 15-20 somites. Numerous mitoses were seen

in the cells of the embryos; we did not find any apparent disruptions on the morphological and histological levels. A small part of the embryos continued to develop and reached the stage of eye pigmentation, but also died shortly.

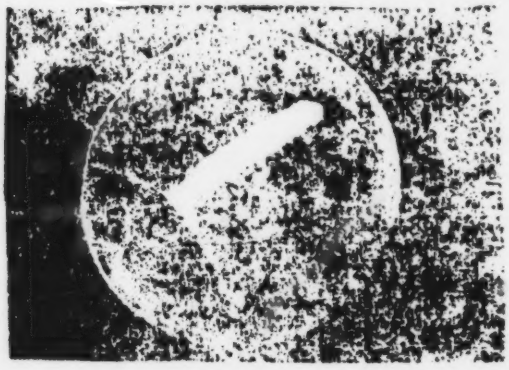

Fig. 2. Yolk-plug stage, during which, at a temperature of 11.6°, the development of most of the embryos ceased. Eggs were incubated in conjuction with water replacement.

At a temperature of 10.0°, only 0.5% of the alevins that hatched were outwardly normal, the remainder had various defects. The duration of the entire incubation period was 43-45 days. During development, the mortality rate of the embryos prior to hatching was 18%. It was greatest at the time of hatching - 75%.

The results of experiments with water replacement during the incubation of Atlantic salmon eggs at other temperatures at which alevins were obtained, are given in Table 4. A comparison of the results of the incubation of the eggs at various regimes showed that the worse incubation indicators are /111 observed at a temperature of 3.1° and lower and at 9.0°; incubation at a temperature range of 4.8-6.2° produces fully satisfactory results. The data in Table 4 are divided into two groups - according to the years in which the eggs were collected, and they have to be compared on the basis of the regimes of a single year.

At a temperature of 6.2° the survival rate of the embryos in the various placements was 95.0-98.2%. The incubation period in this case continues for 79-81 days. The subsequent retention of the alevins in the same regime for approximately one month prior to the beginning of exogenous feeding showed that the vast majority of the alevins were viable (over 90%), and they absorbed the

Table 4

Survival rate of embryos during incubation period, age, size and weight of one-day-old Atlantic salmon alevins, obtained at different temperatures with replacement of recirculated water

Year of collection of egg lot	Incubation temperature, °C	Age at time of fixation, in τ_s	Survival rate, %	Body length of alevin, mm	Weight of alevin, mg	Weight of yolk, mg	Total wieght, mg	Yolk residue in total weight, %
1974	6,2	280	98,2	15,8±0,16	21,7±0,72	51,3±0,89	73,0±1,15	70,3
	4,8	295	94,5	—	22,5±0,50	47,5±1,00	69,6±1,20	68,3
	3,1	288	69,3	15,4±0,11	16,6±0,32	47,8±0,96	64,4±1,01	74,2
1975	9,0	290	86,6	14,6±0,18	16,9±0,37	61,7±1,18	78,6±1,22	78,5
	5,0	290	96,5	15,5±0,15	20,2±0,77	65,3±1,55	85,5±1,71	76,4
	2,0	285	23,4	13,5±0,23	17,8±0,58	64,0±1,68	81,8±1,78	78,2

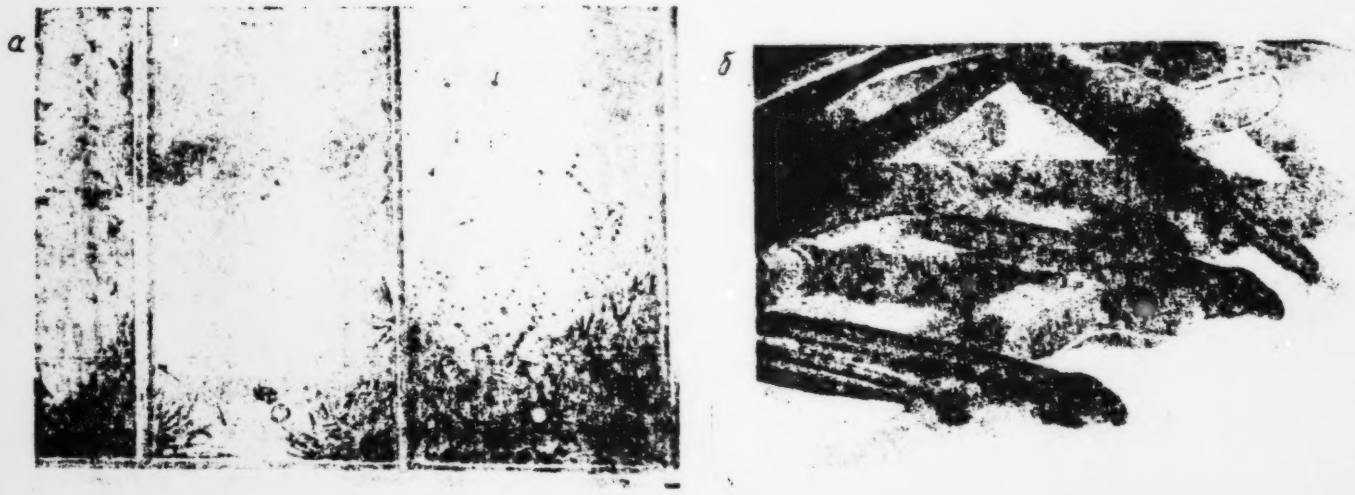

Fig. 3. Normal alevins of Kola Atlantic salmon (in middle compartment), obtained in an incubator with renewal of recirculated water at a constant temperature of 6.2° (a); same alevins at a greater magnification (b)

yolk normally (Fig. 3a, b). Approximately 200 alevins were set apart /112
for external feeding, and this was done with virtually no loss.

There was also a good rate of survival of the embryos at a temperature of 4.8°, and in terms of yolk and body weight the alevins did not differ significantly from that of the alevins from the previous regime. On the basis of these results we can assume that a temperature of 4.8° also provides good conditions for the incubation of Atlantic salmon eggs.

The worse survival rate was obtained when the eggs were incubated at a temperature of 3.1°. In addition, one-day-old alevins weighed less than those that were obtained from the eggs of the same lot in other regimes. These data indicate that embryos that developed at a temperature of 3.1° are suppressed. The cause of suppression is not associated with the large load in the incubator since the expenditure of water per embryo is even greater here than at a temperature of 6.2°.

The next lot of eggs, collected from the Kola River in 1975, developed at temperatures of 2.0, 5.0 and 9.0°. Development at 9.0° took place without visible defects prior to the beginning of eye pigmentation. The first deviations from the norm were found only in the period of the vascularization of the yolk sac. The yolk vein and the entire vascular network of the yolk sac of the embryos was less developed, and the number of vessels and capillaries in the embryos at a temperature of 9.0° was smaller than in normal embryos. Slight mobility was characteristic of the embryos that were extracted from the membranes in late stages of development. However, mortality was low for the period of embryonic development: only 2% died before the beginning of hatching, and another 11.4% during hatching. Incidentally, it was easy to identify the embryos that were dying, even before hatching: they had weakly pigmented eyes, and the yolk sac was speckled with white "grains" of coagulated protein. The alevin yield was 86.6%; however, the alevins were smaller in length and weight than the

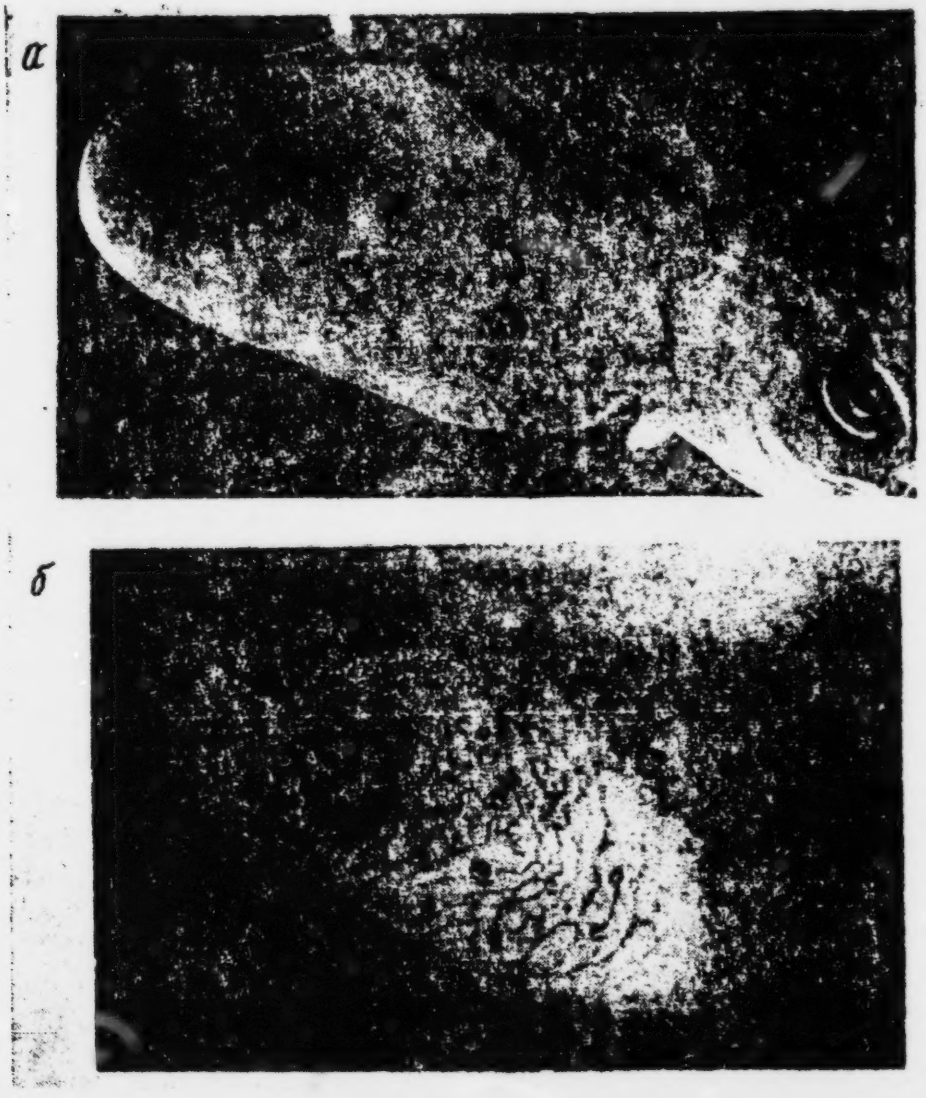

Fig. 4, Alevins of Kola Atlantic salmon which lacks the capillary-vascular system of the yolk sac (a). Incubation conditions: temperature - 2.0°, with replacement of recirculating water

Alevins of Kola Atlantic salmon with a normally devleoped capillary-vascular system of the yolk sac (b). Incubation conditions: temperature - 5.0°, without replacement of recirculating water

alevins from a temperature regime of 5.0° (see Table 4). Further retention and the attempts to transfer such alevins to external feeding revealed the non-viability of most of them.

Incubation of the eggs at a temperature of 2.0° resulted in the development of defects similar to those we observed at 9.0°. Until the beginning of eye pigmentation, this group of embryos developed normally. Subsequently, defects appeared which were connected with the vascularization of the yolk sac, but they were more distinct than at an incubation regime of 9.0°. At 2°, we observe an entire block of the process of yolk-sac vascularization (Fig. 4a). Normally, the yolk vein begins to form simultaneously with the appearance of the first grain of melanin in the eye, and it gradually spreads throught the surface of the yolk with the formation of a vascular-capillary network (see Fig. 4b). In this incubation regime, the yolk vein does not form at all in many embryos. Only isolated bloody lacunae are formed, but they do not unite to form a single blood network, so that there is no circulation of blood in the yolk sac. No vascular system is formed by the end of incubation, and in the remaining vessels there are very few or almost no cellular elements of blood, and only plasma circulates within them. However, such larvae are capable of hatching, and they retain a certain mobility. Other types of defects are also observed in the embryos: distortion of the axial section and irregularity in the form of the distal segments. In comparing the size of the alevins obtained at a temperature of 2.0 and 5.0°, it is seen that the latter surpass the former in this characteristic as well (see Table 4). Incubation of salmon eggs at a temperature of 5.0° is actually a repetition (earlier, 4.8°), and again, according to all of the criterion used, /113 the development of the embryos at this temperature proceeded normally.

Neva salmon. Experiments were set up at four different temperatures for eggs from a single female (Table 5). The survival rate of the embryos at the two extreme regimes - 10.0 and 4.0° - was lower than at the two middle regimes -

Table 5

Embryo survival in incubation period, age, size and weight of alevins of Neva Atlantic salmon obtained at different temperatures with the replacement of recirculating water and preserved at the time of hatching

Temperature in incubator °C	Age during fixation in °s	Survival rate, %	Body length of alevin, mm	Weight of alevin, mg	Weight of yolk, mg	Total weight, mg	Yolk residue in total weight, %
10,0	320	15,8	14,4±0,48	20,5±1,90	121,0±1,90	141,6±2,50	85,4
7,0	315	98,5	17,8±0,08	31,5±0,74	108,7±1,08	140,2±1,31	77,0
4,8	310	95,1	18.5±0,12	30,2±0,56	100,8±0,88	131,2±1,04	76,8
4,0	306	76,6	15,2±0,28	18,1±0,38	111,9±1,06	130,0±1,17	86,1

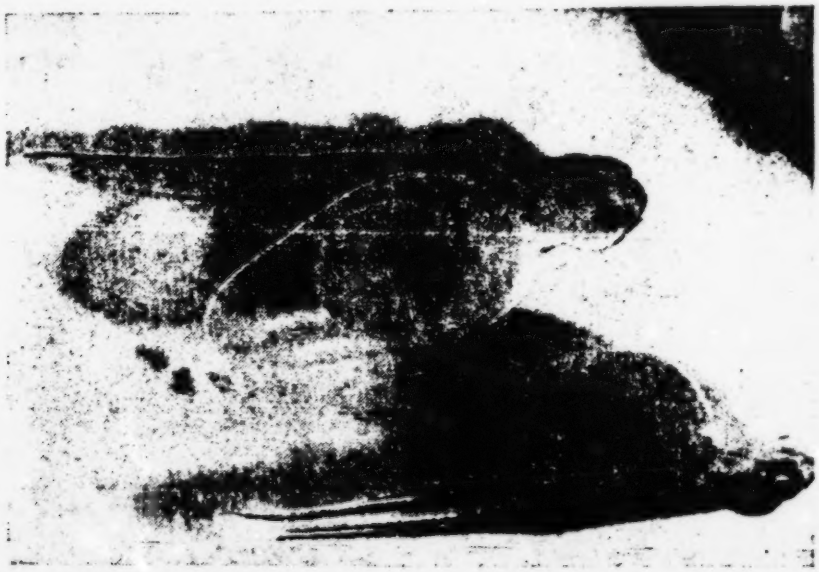

Fig. 5. Alevins with edematous yolk sacs after egg incubation at a temperature of 7.0° with replacement of recirculating water.

7.0 and 4.8°. The results of egg incubation at 7.0 and 4.8° were similar both in the survival of the embryos and in size of one-day-old alevins. The small differences observed were statistically insignificant. The proportion of the residual yolk is also the same in both groups of alevins. Attention is drawn to the fact that the total weight of the alevins from the two warmer regimes is approximately 10 mg greater than that of the two colder regimes.

Fig. 6. Normal Atlantic salmon alevins, obtained at a temperature of 4.8° with replacement of recirculating water

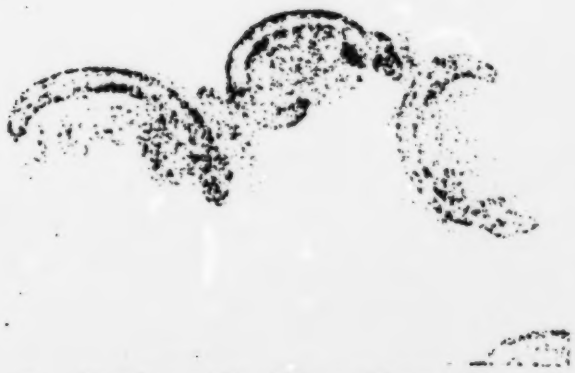

Fig. 7. Alevins with deformations after the incubation of embryos at a temperature of 4.0° with the replacement of recirculating water

We traced the development of part of the alevins obtained at 7.0 and /114
4.8° while holding them at the same temperature (7.0°) until the beginning of external feeding. Practically all (98.5%) of the alevins obtained in the incubator at 7.0° died during retention. The greatest defect observed in these alevins was the great accumulation of fluids in the yolk sac resulting in edema (Fig. 5). The best results were obtained after the retention of the alevins that were obtained in an incubator at 4.8°. This is also seen by the degree of survival (72.8%) and by the external outline of the yolk sac: its oblongish (triangular) form is usually an indication of the healthy state of the alevins (Fig. 6).

At temperature regimes of 4.0 and 10.0°, the incubation of eggs from the same female prooved to be worse in terms of not only the survival rate of the embryos, but also the condition of the surviving alevins: the body weight of the alevins was almost 1/3 less than the body weight of corresponding alevins from temperature regimes of 7.0 and 4.8°. In addition, there were numerous developmental abnormalities, especially at a temperature of 4.0 (Fig. 7). As we can see, in these conditions of incubation, the temperature of 4.0° as opposed to 4.8° cannot ensure the normal development of the embryos.

Incubation of the eggs of Atlantic salmon /115
without replacement of recirculating water

All of the experiments in this series were set up for three years at temperatures ranging from 1.0 to 9.1°. The results were found to be considerably different from those obtained in the variant of experiments with water replacement.

In 1976, we obtained Atlantic salmon eggs from three females, mixed and fertilized by three males, and they were incubated at two temperatures. At 8.0°, the interval between the appearance of successive pairs of somites in the embryos was 280 min, and the entire incubation period lasted 56-58 days. Observations of the course of development of the embryos showed no deviations from the norm during the entire incubation period. The appearance of the successive

stages and the beginning of hatching occurred at the calcuated times. The /116
mortality of the embryos for the entire period was 13.1%. One-day-old alevins of Kola Atlantic salmon were normal in terms of length and weight (Table 6). Let us note that the expenditure of water for incubation per embryo was only 14 mL.

Table 6

Survival rate of embryos during incubation, age, size and weight of Atlantic salmon alevins, obtained at various temperatures without the replacement of recirculating water, at the peak of hatching

Year of collection of egg lot	Incubation temperature, °C	Age at time of fixation in °s	Survival rate, %	Body length of alevin, mm	Weight of alevin, mg	Weight of yolk, mg	Total weight mg	Yolk residue in total weight, %
1976	8,0	295	86,9	16,0±0,14	16,6±0,39	56,9±2,15	73,2±2,18	77,3
	1,0	315	87,6	16,7±0,20	17,9±0,87	50,5±3,03	68,5±3,15	73,7
1977	7,2	296	97,0	15,9±0,08	13,6±0,22	73,8±1,26	87,4±1,28	84,7
	5,0	300	90,3	16,6±0,10	14,1±0,55	51,2±0,58	65,3±0,80	78,4
	2,7	327	97,3	18,3±0,10	24,9±0,40	50,8±0,90	75,7±1,00	67,1
1978	9,1 (I)	290	91,8	16,2±0,07	14,8±0,21	58,8±0,68	73,6±0,71	79,8
	9,1 (II)	290	89,0	15,9±0,08	13,8±0,20	53,5±0,79	67,3±0,82	79,4
	4,0 (I)	310	95,3	17,5±0,15	21,4±0,49	53,4±0,53	74,8±0,72	71,3
	4,0 (II)	310	93,0	17,2±0,10	20,0±0,55	41,2±0,72	61,2±0,91	67,3

Note: First and second placements are indicated in parentheses

A portion of the alevins (150) were set aside for the conversion to external feeding: they were held at this same temperature. Supplementary feeding began fairly late (1.5 months after hatching), when the yolk was very small. Seventy-nine alevins (53%) survived until the yolk was completely absorbed.

In the experiment in which the temperature was kept at 1.0°, good results were also obtained. The interval between the appearance of successive pairs of somites was 1020 min. Development at all stages of embryogenesis slowed down in proportion to this segment, and the relative duration of the observed stages was the same as at other temperatures. The exception was the beginning of the

hatching of alevins (see Table 3), which was delayed somewhat, judging by the age in units of τ_s. Chronologically, hatching began on the 217-218th day, it peaked after the 224-227th days and ended on the 239th day. Converted to relative units, the beginning of hatching occurred at the age of 308-309, the peak - at 316-320 and the end - at the age of 337 τ_s. At higher temperatures, the emergence of alevins began at the age of 282-290 τ_s. Mortality during incubation at 1.0^0 was 12%. During hatching, virtually no embryo deaths were observed: the rate of mortality was only 0.35%. The expediture of water for 7 thousand eggs, taking into account the additions, was 155 L, or 22 mL per embryo. The alevins, as we can see from Table 6, were larger than similar alevins at 8.0^0. One hundred alevins were set aside for further observation. A month after the temperature was brought to 9^0, all of the alevins began to take food (Daphnia, and, later, even small moths). The rate of mortality among the alevins was 20%.

The next series of experiments, in which the eggs of Atlantic salmon were incubated under conditions of non-renewable recirculating water, was set up at temperatures of 2.7, 5.0 and 7.2^0. As in the previous experiments /117 at temperatures of $4.8\text{-}5.0^0$, incubation of the eggs in this variant at the same temperatures ensured the normal course of development without deviations over the same period of time. The rate of survival was fairly high although one-day-old alevins were relatively small (see Table 6) - but this was apparently due to the small size of the eggs of Kola Atlantic salmon.

At a temperature of 7.2^0, the duration of embryogeny from fertilization to the beginning of hatching is 65-66 days. Hatching peaked 70 days after the beginning of incubation and terminated 78 days after incubation. The rate of mortality for the entire period was only 3%. The alevins virtually did not differ in length and weight from the alevins obtained from the thermal

regime of 5.0°; only, the yolk residue was much bigger at 7.2°.

The vast majority of the embryos developed normally at a temperature of 2.7° as well. The span of τ_s was 720 min. Incubation to the beginning of hatching at its peak lasted 158-159 days, and to 100% hatching of the alevins - 168 days. The conversion to relative time shows that here, as at a temperature of 1.0°, the alevins hatched at a later stage than usual. This was apparently due to the much greater length and weight of the alevins as compared to similar alevins from other regimes (see Table 6). The mortality rate of the embryos at 2.7° for the period of embryogeny was 2.5% and another 0.2% of the embryos (from 4200 eggs) died in the hatching period which was over in 10 days. The expenditure of water on the development of each embryo was only 21 mL.

Soon after hatching, almost 2.5 thousand of the alevins which were obtained at 2.7° were delivered to the Northern Lagoda plant and were placed in trays at a temperature of 9°, which was subsequently raised to 14-16°. Over 95% of these alevins were converted over to external feeding. High losses which were noted only in June, two months after the alevins were transferred to the plant, were caused by an outbreak of disease among the plant juveniles. At the end of July, the remaining Atlantic salmon fry - up to 1.5 thousand - were released into a body of water, when their weight averaged approximately 1.5 g.

Finally, in the autumn of 1978, we repeated the incubation of the eggs of Atlantic salmon at temperatures of 9.0 and 4.0°, but now with the circulation of non-renewable water. Eggs which were obtained from two females (first placement) and from one female (second placement) were placed in the incubator. In the first regime, the average temperature was actually 9.1° (±0.1). In this experiment it was determined that the τ_s segment at 9.1° was 250 min, and the entire embryogeny up to the peak of hatching was 49 50 days. On the 50th day of incubation, the yield of alevins from the eggs was approximately 40%, on the the 51st day - approximately 70% and on the 54th day - over 90%. The mortality

during embryogeny for the first placement was 1.9%, for the second placement - 2.7%, and for the hatching period - 9.1 and 5.5%, respectively. The size of the alevins is fairly typical of Atlantic salmon when their development proceeds normally (see Table 6). The alevins showed the capacity for further development since 200 specimens taken from both placements survived until the yolk was completely absorbed, after which they were transferred over to external feeding (the mortality rate was 17%).

At a temperature of 4.0° under conditions of water recirculation without replacement, normal alevins were hatched as well. All of the considered stages began in accordance with the calculated time, including the beginning of the emergence of alevins, which was noted on the 110th day of incubation. However, the hatching period, itself, was extended so that 50% of the alevins hatched only after 121 days of incubation. At this temperature, this corresponds to a relative duration of 315 τ_s. Prior to this, 25 alevins were preserved and measured. Their great length and weight, compared to similar larvae from a /118
thermal regime of 9.1°, are evidently explained by their greater maturity at the time of preservation. The mortality rate in this experiment was also found to be typical for normal conditions of incubation: 4.7% in the first placement, and 7% in the second placement.

DISCUSSION

The results have been presented for the study of the effect of different constant temperatures on the development of the eggs of Atlantic salmon in incubators with recirculating water. It was found that in those experiments in which the recirculating water was renewed, normal development of the salmon was only ensured at a temperature interval of $4.8\text{-}6{,}2^{\circ}$. At these temperatures, the survival rate of the embryos is always above 90%, and the alevins make a good transition to external feeding. A temperature of 7.0° creates conditions for the normal development of the embryos, but probably disrupts the water exchange.

It was found that the total weight of the alevins hatching at 7.0° was greater than of alevins from lower temperature regimes (see Table 5). Since the experimental material was obtained from a single female, it is very unlikely that this difference was the result of individual variability. More likely, the incubation regime at a temperature of 7.0° and higher promotes a greater accumulation of water in the yolk than at lower temperatures. During the further retention of alevins from a regime of 7.0°, they develop "dropsy" (see Fig. 4). The edemism of the yolk sac in alevins after the incubation of the eggs of Atlantic salmon at a high temperature is also described by Peterson et al., 1977).

The incubation regime at a temperature of 9.0° with replacement of the recirculating water causes even more serious consequences: the survival rate of the embryos is lower, the alevins are smaller at hatching (see Table 4), and serious irregularities in the formation of the blood-carrying system of the yolk sac are already found in the middle of embryogeny. At higher temperatures, almost all of the embryos die, either at hatching (10.0°) or at the beginning of organogenesis (11.6°).

The results of the incubation of salmon eggs at a temperature of 4.0° and lower in the variant of experiments with the renewal of the recirculating water should be regarded as unexpected. As we know, salmon eggs develop in this temperature zone in natural conditions. In our experiments with the periodic replacement of water, the retention of the eggs at these temperatures was accompanied by an elevated mortality rate, reduced alevin length and weight, and the development of many defective individuals. The lower the temperature was kept in the incubator, the lower the survival rate of the embryos (see Table 4 and 5) and the greater the defects observed in the embryos.

Thus, the results of the study of the development of the eggs of salmon at constant regimes of different temperature, when the recirculating water

in the incubator is periodically partially renewed, showed that the embryos develop normally only within a narrow temperature range - at 4.8-6.2°. At other temperatures which are higher and, particularly, lower, we did not manage to obtain viable alevins. This fact may indicate that the temperature range of 4.8-6.2° is the most suitable for the development of salmon embryos.

The results of the other series of experiments, with non-renewable water for the entire incubation period, were more unexpected and make this variant a promising one for practical use. At all temperatures of the investigated range - from 1.0 to 9.1° (7 different regimes) - the survival rate of the embryos was high; at some temperatures, hatching was accomplished with almost the ultimate level of survival; alevins were viable even in /119 extreme regimes, and the majority of them tranferred over to external feeding. We shall note that at periods of incubation of 2 to 8 months at different regimes the expenditure of water per embryo was only 14-28 mL, which is many times less than that recommended for the incubation plant at fish hatcheries (Yandovskaya and Kazakov, 1974). In spite of this, the range of temperature tolerance was found to be broader here than in the experiments with the partial replacement of the water circulating in the incubator. In fact, we have not yet managed to establish the limits of tolerance of the embryos to the temperatures of development in these zones, in spite of the fact that we have already investigated a considerable temperature series - from 1.0 to 9.1°. Not one of the incubation regimes studied showed an appreciable number of embryos with developmental defects, and most of the alevins were easily transferred over to external feeding and were capable of further development, as was the case with alevins from the regime of 2.7°.

When we evaluate the two experimental variants, we have the impression that more favourable conditions of embryo incubation are produced with a completely closed water supply (without water renewal) because

of the formation of micro-conditions in the environment with which the embryos are in constant interaction. This micro-environment may contain, in addition to the metabolites, special substances which regulate development. It is interesting that the longer the incubation period, the lower the mortality during the period of hatching. This period is often characterized by an elevated mortality rate (Bonnet, 1939; Hayes, 1949). In our experiments, the mortality rate during this period at a temperature of 2.7 and 1.0° was only 0.20-0.35%, and at a temperature of 9.1° - 5.5-9.1%. When part of the water is replaced, the equilibrium which is established between the medium and the embryos is disturbed, having a negative effect on the course of development of the embryos. Evidently, a constant micro-environment is very important for the realization of normal embryogeny. This phenomenon may also be important for adult fish: we are well acquainted with the rule which prohibits sudden water displacement in an aquarium, since this often leads to the death of its inhabitants (Makhlin, 1975).

It is necessary to discuss briefly the results of the incubation of salmon eggs at the temperature of 1.0° since this is the first experiment on the incubation of the embryos of this species at such a low controlled temperature. It is believed that salmon embryos in early and late stages of development, which take place in autumn and spring in nature, have poor tolerance to low temperatures. P.P. Ivanov (1949) set up an experiment in which salmon eggs were kept in non-flowing water at a temperature of 0.2-0.5°. Embryos developed poorly, and they died during the hatching period. N.I. Yandevskaya (1976) believes that if after fertilization, salmon eggs occur at temperatures not above 1.5-2.0°, then subsequently, in most cases, alevins hatch which are incabalbe of transferring to external feeding. The author hypothesizes that they have a defect in the development of the digestives system. However, our experience did not corroborate this hypothesis. Alevins which were obtained after almost 8 months of incubation

were transferred to external feeding, which indicates the normal development of their digestive system. Nor should we disregard the notion that the conditions of a fully closed water circulation may be better than natural conditions, and that they broaden the temperature range at which salmon develop normally. We know from ecology that the range of tolerance to one ecological factor can be narrowed if the conditions of another are not optimal, and vice versa (Odum, 1975). Evidently, this same principle can also be applied to the /120 interpretation of the fact that during the development of salmon in incubators with partial water replacement, the range of temperature tolerance narrows to 5.0-6.0° (4.8-6.2°).

In conclusion, it should be repeated that temperatures of 5.0-6.0° ensure the normal development of the embryos in all variants, and it can be assumed that this range is the optimal zone in the temperature scale for the incubation period of Atlantic salmon development. Let us note that the zone of 5.0-6.0° occurs approximately in the middle of the range of tolerance or standard reaction. The latter usually has its maximum in the middle of the of the range. Using another method, Canadian investigators (Peterson et al., 1977) obtained similar data on the optimal temperature of incubation.

An entirely closed water exchange during incubation opens up extremely interesting prospects for establishing an optimal regime for the development of salmon embryos. There is no doubt that this method provides the most economical variant for dealing with the problem of maintaining the required temperature in an incubator. As to the latter, we consider the temperature range of 5.0-6.0° to be the most biologically adequate for salmon embryos. If salmon eggs are not incubated at this temperature, it will be impossible to accelerate (by 1-1.5 years) the development of downstream migrants.

CONCLUSIONS

1. Atlantic salmon eggs develop normally in an incubator which has a closed system of water supply without regeneration or renewal of the recirculating water. The water expenditure per embryo for the entire incubation period is 14-28 mL.

2. In such conditions within a constant temperature range of 1.0-9.1°C, salmon alevins develop normally in an incubator and are capable of transferring to exogenous feeding.

3. The partial periodic renewal of the recirculating water diminishes the temperature range, which allows for the development of viable alevins to 5-6°C.

4. The temperature of 5-6°C, which allows for the normal development of salmon eggs in various variants of incubation, can be regarded as the optimal temperature for the incubation period of this species.

5. The method of incubating eggs with recirculating non-renewable water has promising practical use because of its technical simplicity and economy and the possibility of maintaining an optimal temperature regime for the developing embryos.

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